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Studies in Reptilian Colour Response. I. The Bionomics and Physiology of the Pigmentary Activity of the Chameleon

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II. *Studies in Reptilian Colour Response.* I.—*The Bionomics and Physiology of the Pigmentary Activity of the Chameleon.*

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[Plates 4 and 5.]

Introduction.

The literature of reptilian colour change extends over twenty three centuries, and yet our knowledge of the physiological processes which govern it, and of the environmental factors which bring it about, is to-day far less complete than for the other two groups of vertebrates which possess pigmentary effectors. The cause of this, it would seem, resides in the fact that the colour changing reptiles inhabit pre-eminently the tropical and subtropical regions of the globe, and are not readily available for physiological investigation in the main centres of scientific progress. The history of this subject is peculiar. From Aristotle to the end of the nineteenth century the literature deals almost exclusively with the chameleon, an animal which for centuries has excited the curiosity of travellers in North Africa, and which, in consequence, has acquired a popular reputation that is quite remarkable. Thus the hundred pages which Fuchs (1914) devoted to reptilian colour response contain far more references to chameleons than to all other reptiles taken together. In the present century, with the single exception of the work of Hogben and Mirvish (1928) from this laboratory, no further investigations on the chameleon have been published. Our knowledge of colour change in reptiles has progressed chiefly through the work of Professor G. H. Parker and his many associates. In the New World chameleons do not exist, and consequently the American workers have turned to other lizards, chiefly *Anolis* and *Phrynosoma*. Thus it has come about that most modern workers in this field are relatively unfamiliar with the chameleon, and have tended to overlook the many interesting facts concerning colour change in this animal recorded in the earlier literature.

But there is another, and perhaps more cogent reason for the neglect which the work of the earlier investigators has suffered during the last ten or fifteen years. The discovery of the endocrine control of pigmentary effectors in amphibia, chiefly through the investigations of Hogben and Winton (1922, 1923), served to discredit all the previous work on the alleged nervous co-ordination of colour change in this group. Four years earlier, Redfield (1918) had brought forward evidence (far less conclusive,

it is true) of the control of the melanophores in *Phrynosoma* through internal secretions. REDFIELD'S work undoubtedly gained support, indirect but none the less powerful, from the new discoveries in the field of amphibian colour response. It began to look as if the road to the unification of the theory of pigmentary response in vertebrates lay in the direction of the hitherto unsuspected role of the organs of internal secretion. Two reviews published ten years ago give prominence to REDFIELD'S results to the almost complete exclusion of all earlier work on reptiles. Of the two authors, VON BUDDENBROCK (1924) did concede the possibility of a second, *i.e.*, nervous, co-ordinating mechanism, but HOGBEN (1924) was inclined to question such a possibility, and advanced arguments to show that the experimental phenomena observed by REDFIELD might "arise secondarily through interference with the blood supply." Four years later this author (HOGBEN and MIRVISH, 1928) re-investigated the chameleon. They found that REDFIELD'S conclusions were not at all applicable to this reptile; their own observations indeed offered strong support to the theories of the nineteenth-century investigators. They concluded that, "until the influence of adrenal secretion has been demonstrated in material more favourable for the study of colour response than *Phrynosoma*, it is legitimate to express the doubt that the phenomena of colour response in reptiles provide conclusive evidence of the possibility of defining conditions in which the liberation of adrenaline into the vertebrate circulation in increased quantity takes place." Thus the possibility of a coherent theory of reptilian colour response seemed more remote than ever. No further light on this problem has been forthcoming. Of recent years PARKER (1930, 1932) in reviewing the subject, has been constrained to attempt a compromise. In the earlier review he writes, "in reptiles the two sets of influences, nervous and humoral, appear to be almost balanced, though not enough is known about the colour activities of this group of vertebrates to allow of final judgment"; and in the later, with reference to the work of REDFIELD, and of HOGBEN and MIRVISH he says, "Both these pieces of investigation show satisfactory evidence for these two reptiles of direct nerve control of melanophores similar to that already established for fishes, and in one of these there is much that is indicative of a supplementary humoral influence."

It is evident that the situation is unsatisfactory. The present investigation was undertaken primarily with the object of defining the bionomic aspects of colour change in the chameleon more completely than has been done hitherto. It has led to conclusions of more general interest in connection with the physiology of reptilian colour change.

PART I.

The Influence of Light on the Pigmentary Response of the Chameleon.

It is now well known that the principal environmental factors governing colour change in reptiles are light and heat. In spite of much contradictory evidence in the earlier literature, which has been fully reviewed by BRÜCKE (1852) and by FUCHS

(1914), modern workers are agreed that reptiles are pale in the dark, and darken when exposed to light. This was established for the chameleon by BRÜCKE (1852), for *Anolis carolinensis* by CARLTON (1903), PARKER and STARRATT (1904) and VON GELDERN (1921), for *Anolis equestris* and *Anolis porcatus* by HADLEY (1928, 1929) for *Phrynosoma blainvillei* by PARKER (1906) and for *Phrynosoma cornutum* by REDFIELD (1918). The evidence of DE FILIPPI (1866) and THILENIUS (1897) that *Stellio*, *Varanus*, and *Uromastix* display the opposite reaction, was criticized by PARKER (1906) on the grounds that in their observations temperature was an uncontrolled variable. Much confusion would be avoided if it were agreed to disregard all observations on chromatic reactions to light except those that are based on determinations carried out in a photographic dark room and in strong diffuse daylight at measured room temperatures.

The observation that the illuminated side of a chameleon exposed to the sun is darker than the shaded side was made, according to BRÜCKE, as early as 1651 by DE PEIRESC. BRÜCKE investigated this phenomenon, and showed, by placing a girdle of tinfoil round the body of a chameleon exposed to sunlight, that the darkening of the skin is a localized response, a conclusion which has been subsequently confirmed by BERT (1875), KELLER (1895), and HOGBEN and MIRVISH (1928). REDFIELD (1918) records a similar local response in *Phrynosoma*, and HADLEY (1928) in *Anolis equestris*. CARLTON (1903), however, claimed that illumination of a part of the body of *Anolis carolinensis* caused a general darkening of the whole skin. No attempt has apparently been made to verify this exceptional observation.

Since the skin of the chameleon responds locally to incident light, most previous workers seem to have been satisfied that this mechanism is sufficient to account for the whole range of the chameleon's chromatic response to photic stimulation. Very few have envisaged the possibility that the eyes may also be in some way concerned. BERT (1875) claimed that the removal of one eye caused a pallor on the "corresponding" side of the body, an observation which KELLER (1895) was unable to confirm. BERT further announced that "l'ablation des deux yeux rétablit l'équilbre," a statement which gives no information as to whether blinding does or does not interfere in any way with the chromatic response of the animal. The only other evidence on this point comes from TOMASSINI and CONSIGLIO (1890), who, according to FUCHS,* found that the removal of both eyes had no effect on the colour response of the chameleon. Thus it is clear that no exact information exists concerning the role of the organs of vision in this connection. The present confusion on this issue is well typified by the comparison of the statement of VON BUDDENBROCK (1924) that "Blendung hat keinen Einfluss auf die Färbung der Reptilien," with that of PARKER (1930), who says ". . . if both eyes are incapacitated (the colour play) ceases completely. This peculiarity has been repeatedly demonstrated in the past and is known to be characteristic not only of such simple colour reactions as that of *Fundulus*, the frog, and

* FUCHS was unable to obtain this paper in the original, his reference to it is therefore indirect.

Anolis, but also of the complex ones, as, for instance, those in flat fishes and in the chameleon."

The latter statement, so far as the chameleon is concerned, is certainly erroneous. A blind chameleon displays maximum pallor in darkness, and becomes black in strong daylight. Figs. 9 and 12, Plate 4, show the limits of colour change in such a blind animal. The range of pigmentary response is not reduced by blinding. Nevertheless, the organs of vision do play a very active and hitherto unsuspected part in the chromatic responses of the chameleon.

The most generalized fact about colour response in amphibia, fishes, and even crustacea is that these animals respond to a light scattering background by melanophore contraction, and to a light absorbing background by melanophore expansion.* It is curious that among the modern workers in reptilian colour change only one has sought for a similar background response in reptiles. REDFIELD (1918) observed that *Phrynosoma cornutum* became very dark on black cinders, and very pale on white sand. The reaction in each experiment took several days to reach a maximum. This observation furnishes strong *a priori* justification for the view that the reptiles form no exception to the general rule of the effect of background on the chromatic responses of animals. In the chameleon this expectation has been fully realized.

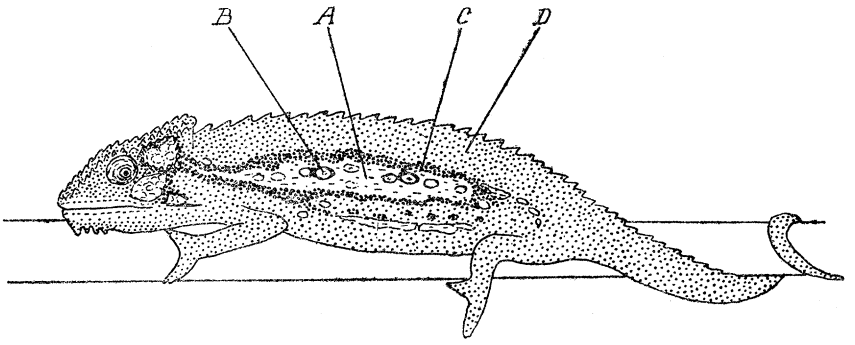

FIG. 1.—Typical dermal pattern of *Lophosaura pumila*. A, "band"; B, "island"; C, "margin"; D, "back."

The dermal pattern.—The animal employed in our experiments was the dwarf chameleon of the Cape, *Lophosaura pumila* (Daud). It has been long known that the chameleon possesses a constant dermal pattern consisting of stripes, patches, and even individual skin tubercles of different colours, within each of which a change from a light to a dark shade may take place (BRÜCKE, FUCHS, PARKER, *et al.*). In the Cape chameleon, although the general pattern is constant for the species, there are considerable variations in detail. The markings on a typical individual are indicated in fig. 1. For purposes of reference we have named the components of the pattern "bands," "islands," "margin," and "back," as shown in the figure. In the condition of extreme pallor

* In accordance with the now well-established usage the terms "expansion" and "contraction" are used to signify respectively the distal and proximal migration of the melanophore pigment.

the " bands " are pale fawn in colour, in exceptional cases they may be quite white, the " islands " are pale grey in some individuals, pale fawn in others, the " margin " is pale grey or pale blue, the " back " is yellow-green. In the intermediate state the " bands " range through orange to brown, the " islands " usually assume dark shades of blue or grey, the " margin " is bright blue and the " back " is green. In the condition of extreme darkening the whole skin becomes black. " Bands " are present in all individuals, though they may vary considerably in width. The " back " is fairly constant in all adult chameleons, though it may be somewhat more yellow in some than in others. The " islands " and " margin " are vary variable and may be absent altogether. Further, in many chameleons, large orange tubercles are found arranged in rows in the green region of the skin (*i.e.*, the " back "). These have the same range of colour as the tubercles of the " bands." As a result of such variations a random group of chameleons in any given situation may present a heterogeneous appearance which defies general definition.

The distribution of pigment and chromatophores.—The structure of the chameleon's skin will form the subject of a separate communication. Here we shall record only certain general observations which suggest an interpretation of the colours displayed in the different regions of the dermal pattern. Unstained frozen sections of skin show the distribution of the pigment very clearly. Such sections reveal important differences in the distribution of pigment in the tubercles of different regions of the dermal pattern.

1. *The yellow pigment.*—Underlying the epidermis of the tubercles of the " back " is a uniform layer of yellow pigment. This is totally absent from the tubercles of the " bands " and the " margin."

2. *The orange pigment.*—The same level in the tubercles of the " bands " is occupied by orange pigment which is seen to be clumped together in some tubercles, and spread out in others. There can be little doubt that the cells bearing this pigment are true chromatophores. This pigment is seen also in the large orange tubercles which occur here and there in the green region of the skin.

3. *The " blue layer."*—Underlying the yellow layer in the tubercles of the " back " is a region which by transmitted light is almost colourless, but by reflected light appears blue. It would seem that this is not due to pigment, but is an interference effect. The important point is that this layer is clearly seen in the tubercles of the " back " and of the " margin," and is totally absent from the " bands " and the large orange tubercles.

4. *The " white layer."*—The deeper region of the skin in all tubercles appears dark grey by transmitted light, but by reflected light it is white. This layer extends right up to the orange pigment in the tubercles of the " bands " and in the large orange tubercles. In all other tubercles it underlies the " blue layer."

5. *The melanophores.*—These are seen embedded in the white layer, or in the connective tissue underlying it, in all tubercles. In sections of pale skin all the dark

pigment is in the deeply situated cell bodies of the melanophores. In very dark skin it has all migrated up the peripherally directed cell processes, and forms a dense reticulum which obscures the yellow and orange pigments.

On the basis of these observations the following scheme suggests itself.

Dermal Region.	Colour Range.	Yellow Pigment.	Orange Pigment.	Blue Layer.
"Bands"... ...	Pale fawn, orange, brown ...	Absent	Present	Absent.
"Margin" ...	Grey, blue, dark blue ...	Absent	Absent	Present.
"Back"	Yellow, green, dark green ...	Present	Absent	Present.

Thus it appears that the green colour of the tubercles of the "back" is due to the superposition of yellow pigment upon the blue "layer," the blue colour of the margin is due to the "blue layer" in the absence of yellow pigment, the orange colour of the "bands" is due to the orange chromatophores and the absence of the "blue layer" from this region. Maximum darkening is of course due to extreme distal migration of the melanophore pigment in all tubercles.

It is a fact of considerable interest that the bright colours seen in adult chameleons are not present in young ones. As is well known, *Lophosaura pumila* is a viviparous species. Many births have occurred in the laboratory. The young ones are invariably grey in colour, becoming almost white in conditions of extreme pallor, and black when maximally dark. No green, orange or blue colours are present, although a little brownish-yellow has been seen in the "bands" of some individuals. The pigments appear only when the chameleons are nearly full grown. The average length of an adult female, from the snout to the tip of the tail, is about 15 cm. The males are somewhat smaller. The average length of new-born chameleons is 4·2 cm. Females 12 cm. in length are still unpigmented, males of this size are already beginning to show some green and yellow colour. These observations suggest an interesting speculation concerning the more primitive phylogenetic origin of melanophores as compared with other pigment cells. They are in agreement with the observations of Leydig (1873) on *Anguis fragilis*, and of de Filippi (1866) on *Stellio cancasicus*.

It may be mentioned parenthetically that although females of *Lophosaura pumila* appear to be more numerous than males, the sex ratio is not as abnormal as Hogben and Mirvish (1928) believed. The following table indicates the proportion of the sexes.

On April 18, a female gave birth to 18 young in the laboratory between the hours of 9.15 a.m. and 12.30 p.m. She was observed the whole time. The litter contained 7 females and 11 males. The males are easily distinguished by squeezing the ventral surface of the tail just posterior to the cloaca, which causes the two penes to protrude.

Records were not kept earlier than April 7, but among the several dozens of chameleons that were used for experiments during the months November to March, males were quite common. We are unable to explain the statement of HOGBEN and MIRVISH that of about 200 animals collected for their experiments not more than half a dozen were males.

Date.	No. of animals collected.	Males.	Females.
April 7, 1933	24	9	15
April 13, 1933	25	8	17
April 24, 1933	10	6	4
May 5, 1933	11	4	7
Total	70	27	43

The background response.—Preliminary tests convinced us that chameleons respond to background in the usual manner. On a white background they are light coloured, on a black background they are dark. It seemed advisable, however, in view of the complexity of the dermal pattern and the many colours involved, to attempt a more exact method of recording than is possible with the use of the adjectives "pale" and "dark." Accordingly, colorimetric scales of appropriate shades were adopted for each region of the dermal pattern, and the "bands," "islands," "margin," and "back" were separately recorded. The records showed that in general the whole dermal pattern became uniformly paler or darker. Because of this fact, and also because individual variability in the "bands," "islands," and "margin" made it difficult to fit a large number of animals into the colorimetric scheme, this complicated method of recording was ultimately abandoned in favour of the simpler one of recording the colour of the "back" only. For this purpose we have adopted the procedure devised by HOGBEN for recording the melanophores of Amphibia, and have assigned numerical symbols to five stages of progressive darkening of the "back" region of the skin. The scale employed was the following :—

Index ..	1	2	3	4	5
Colour ..	Yellow	Pale Green	Medium Green	Dark Green	Black.

The range of the responses to background is well seen in figs. 6 and 7, Plate 4. These should be compared with fig. 9, which shows a blinded chameleon exposed to the same conditions. The photographs show that in the blind animal illumination of the skin causes darkening irrespective of the background on which the animal is placed, while the normal animal darkens only on a light absorbing background, but remains pale on a light scattering one.

For reasons that will appear later it was desired to obtain data which would show the time relations of the responses to background. There are two ways in which this could be done. Either animals could be changed from a white background to a black one and *vice versa*, and their colour indices recorded at intervals sufficiently frequent to give points on the steep part of the curve, or all the animals could be allowed to become adapted to total darkness (maximum pallor), and could then be brought into light on black or white backgrounds and their indices recorded at intervals measured from the instant of illumination. The latter procedure is to be preferred for the following reasons: the condition in darkness is the most uniform and the least subject to individual variation, and provides a convenient point of departure for any series of observations. The time relations of blind and normal animals can only be derived from observations on dark-adapted animals, since darkness is the only condition to which these two classes of animals respond in the same way. In darkness external photic stimulation is absent, and the change from an unstimulated state to a stimulated one is likely to be easier to interpret than the change from one form of stimulation to another.

The animals were placed into individual black and white dishes with glass covers. They were left in the dark room for 30 minutes, an interval more than sufficient to allow

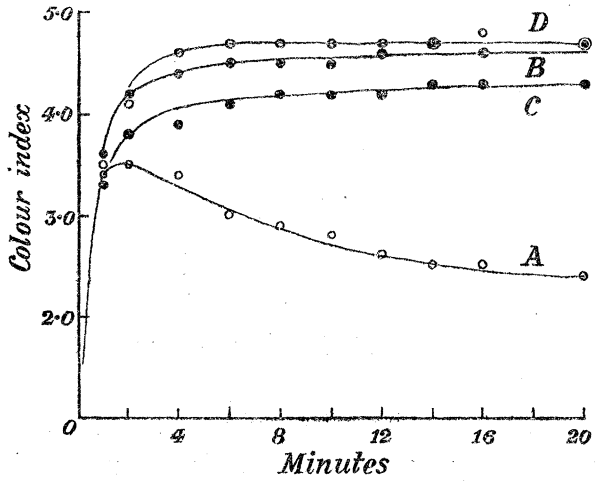

Fig. 2.—Time curves of blind and normal animals brought from darkness into *daylight* in black and white containers. Temperature 13-17° C. Curve A. Normals on white, 25 animals. Curve B. Normals on black, 25 animals. Curve C. Blind on black, 15 animals. Curve D. Blind on white, 15 animals.

them to attain a state of maximum pallor. They were then carried into the laboratory and placed near a window in strong diffuse daylight. Recording was begun immediately. Chameleons darken so rapidly on exposure to light that, when ten animals were used for an experiment it was impossible to record their initial condition accurately. Since it could be seen at a glance that they were all either yellow or pale green, the index 1·5 has been plotted as the initial point for all curves. Fig. 2 shows the equilibration

of normal and blind animals on black and white in daylight. The records for normal animals were somewhat more variable than for blind ones. For this reason larger numbers of normals were used. The curves show that blind animals darken rapidly on exposure to light, and attain a condition of extreme darkening in about six minutes. Normal animals on black give a curve very similar to that of blind animals. On white they darken rapidly during the first minute at about the same rate as blind animals or normals on black, and then slowly become pale again, attaining a steady level only after about 15 minutes. The theoretical implications of the peculiar shape of this curve will be discussed at length in Part IV.

The darkening of the skin in animals brought from darkness into daylight is usually uniform over the whole integument, excepting that the ventral surface of the body may be somewhat paler. This implies that melanophore expansion and contraction occurs uniformly throughout the whole dermal pattern. Occasionally, however, animals have been noticed in the laboratory cages in which this was certainly not so. These animals presented a strikingly mottled appearance. The skin of the back was divided into a segmental series of blocks of alternately pale green and dark green colour the bands were almost white with very dark islands, and the margins were black. We have called such a condition "contrast." No information concerning the stimuli that call forth this appearance exists. Such differential nervous control of the melanophores implies a mechanism of co-ordination more elaborate than any that has yet been described in connection with pigmentary effector activity.

It is generally stated (*e.g.*, VON BUDDENBROCK, 1924) that, within limits, the intensity of illumination is of no importance in the response of fishes to background. In amphibia, HOGBEN and SLOME (1931) have shown that on a white background the melanophores of *Xenopus lævis* are slightly more contracted in dim light than in bright light owing to the antagonistic primary reactivity. Since, in the chameleon, the darkening of the skin in response to direct illumination is even more pronounced than the primary response in *Xenopus*, it was to be expected that chameleons would show a more extreme pallor on a white background in dim light than in bright light, and a more extreme darkening on a black background in bright light than in dim light. Accordingly, determinations were made in the photographic dark room with artificial illumination from a 400-candle power gas-filled lamp totally enclosed in a frosted shade, hung at a height of 10 feet above the dishes containing the chameleons.

The results were quite unexpected. Fig. 3 shows the equilibration of normal and blind animals on black and white backgrounds under such conditions. Three important conclusions emerge from a consideration of these results. (*a*) The intensity threshold is significantly lower for the background response than for the response to direct illumination of the skin. This is shown by the relation of the curves for blind animals to the curves for normals. (*b*) On black in lamplight darkening was even more extreme than in daylight (*cf.* fig. 2). (*c*) *Normal animals on a white background in lamplight responded with a very pronounced darkening.* This response, as the curve shows, was

slower than the darkening of normals on black, or than that of blind animals exposed to daylight (fig. 2). It was also much more variable; some animals were very dark after two minutes' exposure, others darkened only after 20 or 30 minutes, a few remained pale even after 60 minutes.

The conclusion is unavoidable that in weak light of the kind employed in this experiment chameleons become dark on both light absorbing and light scattering backgrounds. This response is effected through the eyes and has nothing whatever to do with the response to direct illumination of the skin as the two curves for blind animals in fig. 3

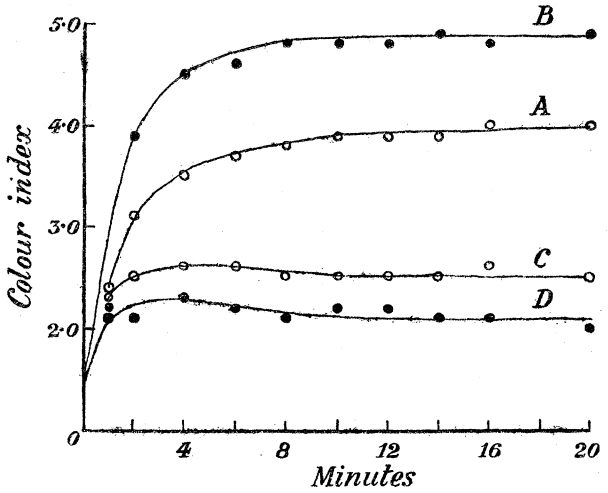

FIG. 3.—Time curves for normal and blind animals brought from darkness into *lamplight* in black and white containers. Temperature 13-15° C. Curve A. Normals on white, 30 animals. Curve B. Normals on black, 15 animals. Curve C. Blind on white, 15 animals. Curve D. Blind on black, 15 animals.

clearly show. The lamplight was not strong enough to cause more than a very slight darkening (change from yellow-green to pale green) in the skin of the blind chameleon.

As far as we are aware nothing comparable to such a reversal of the white background response in weak light has been recorded for any animal. Yet in the colour responses of the chameleon this phenomenon is of great importance. Many other observations have confirmed this opinion. Chameleons were frequently seen to assume a very dark coloration on the cement floor, under the bench. The explanation that at first suggested itself was one which has been offered for the darkening of reptiles on numerous occasions by many authors, namely, that this was a low temperature effect due to the cold concrete floor. After the discovery of the reversal of the white background response in lamplight, it became clear that this phenomenon had no connection with temperature, but was due to the low intensity of the light under the bench. Such an interpretation can be established with absolute certainty by comparing the response of intact with that of blind animals in any given situation. It is inconceivable that the chromatic reactions of blind animals with respect to temperature should differ in any way from the reactions of normal ones. Blind animals on the cement floor under the bench

were quite pale. It thus appears that the reversal is due to low intensity rather than to differences in the spectroscopic quality of the light, as might have been inferred from the fact that the response was first observed in lamplight.

The phenomenon can be most strikingly demonstrated in the following way: the experiment is performed in a room whose windows are in one wall, along the whole length of which there is a laboratory bench just under the windows. A chameleon is put into a white enamel dish. The dish is placed on the bench. After a few minutes the animal is pale. The dish is put on the floor under the bench. After a few minutes the animal has become dark. The observation can be repeated an indefinite number of times. The degree of darkening, however, is greater with some individuals than with others.

Brücke (1852) quotes an observation of long ago, which can now be interpreted in the light of the present conclusions. The quotation is from "Voyages de Monsieur de Monconys," Paris, 1695. "J'observai comme mon Chaméléon, qui etait vert, entrant dans ma chambre; l'ayant mis sur une feuille de papier blanc devient noir, ce que j'attribue à la chandelle, parce que l'ayant remis à l'ombre il reprît la couleur verte. . . ." The darkening of the chameleon in this case was due to the white background illuminated from a candle.

The responses to background are illustrated in the accompanying photographs. Fig. 7, Plate 4, shows a chameleon on a white background in strong diffuse light. Fig. 6 shows the same animal on a black background, fig. 8, on a white background in lamplight, fig. 11, the same animal in darkness. These should be compared with the photographs of a blind animal in daylight, lamplight, and darkness, figs. 9, 10, and 12. Inspection of these photographs will make it sufficiently evident that observations of the pigmentary responses of chameleons under different conditions of illumination are valueless if the nature of the environment is not defined. Much of the contradictory evidence that is to be found in the literature may be thus explained.

Part II.

The Effect of Temperature on the Pigmentary Response.

High Temperature.—Concerning the effect of heat on reptilian chromatophores there is general agreement. High temperatures cause contraction. Brücke (1852) found that at 33° C. chameleons in the light become pale as quickly as at 16° in the dark. Similar observations were made on *Anolis* by Parker and Starratt (1904) on various lizards by de Grijs (1899), on *Phrynosoma blainvillei* by Parker (1906), and on *P. coruntum* by Redfield (1918). The average temperature at which reptilian chromatophores change from the expanded to the contracted state in the light seems to be somewhat above 35° C.

Our own observations are in agreement with this principle. Chameleons placed in an embedding oven lined with black paper and provided with a glass lid were dark

at 18° C. When the temperature was raised to 40° C., they became maximally pale in 3–4 minutes. When dropped into water at 37° C. the animals turned pale in one minute and when taken out regained an intermediate colour. In cold water the pallor did not occur.

Under natural conditions the response to high temperatures commonly occurs on hot sunny days. Thus blind and normal animals which were placed on a lawn in the hot sun turned maximally pale in five minutes. A chameleon set out in the sun in a black dish was dark for two or three minutes, and attained maximum pallor at 5 minutes. This animal made violent efforts to escape from the dish. A thermometer exposed to the sun rose to 40° C. after 2 minutes.

Chameleons succumb rapidly to exposure to hot sunshine, and are usually found lurking in the shade on hot days. A cage containing two dozen chameleons was accidentally left out of doors overnight in such a position that the animals were unable to escape the direct rays of the sun when it rose in the morning. When discovered at about 9 a.m. they were all dead. It was a particularly hot summer day.

Low temperatures.—The statement that low temperatures cause expansion of reptilian chromatophores is made by von Buddenbrock (1924), Hogben (1924), and Parker (1930). Compared with the data on the effect of high temperatures, the evidence on this point is singularly unconvincing. The first exact determinations were those of Parker and Starratt (1904) on *Anolis carolinensis*. They showed that lizards which had become brown in the light did not become green when placed in darkness at 10° C. They omitted, however, to record the duration of exposure to darkness. This was an unfortunate omission in view of the opinion expressed by Fuchs (1914) that low temperatures merely reduce the velocity of the proximal migration of the pigment in the absence of light. More conclusive was their observation that green animals when introduced into a dark box at 10° C. became brown in about 18 minutes. But its value as evidence is considerably diminished by the statement that "this record . . . can be regarded as only approximate."

Further evidence on this question was advanced by Parker (1906), Redfield (1918), Hadley (1929), and Smith (1929). The evidence of Parker on *Phrynosoma blainvillei* is contained in the following statement: "At 15° C. in bright diffuse daylight, the lateral scales had very pronounced dark centres, much as at 19° C. under similar illumination. These dark centres were partly but not completely lost when the animal was kept in the dark at 15° C. As these centres usually disappeared completely at 19° C. in the dark, it is clear that a low temperature favours a distal position for the pigment." The evidence of Redfield is based on an experiment in which *Phrynosoma* was allowed to become dark in diffuse light at 16° C. The light was then excluded. "After one half-hour the melanophore pigment was still *expanded*" (p. 281). On the previous page, in describing the responses to light and darkness, Redfield says, "The rapidity with which these changes occur in either direction varies from ten minutes to one half-hour . . ." It seems, therefore, that the failure of an animal to change from

a dark to a light condition during 30 minutes exposure to darkness is not unusual at any moderate temperature. REDFIELD also investigated the effect of the local application of low temperatures by means of a nozzle through which ice water was running, which was brought into contact with one side of the animal. The results obtained were very significant. When the nozzle (the temperature of which was 10·5° C.) was applied to a pale animal in the dark, *no change was observed in* 30 *minutes*. When applied to a dark animal in the dark, " after *six minutes* the melanophore pigment of the animal had contracted, as might be expected in the dark. The melanophore pigment of the chilled portion of the skin *did not contract in twice that time*." [Italics inserted.] These results appear to be more in agreement with FUCHS' view (see p. 38) than with PARKER's. In none of these experiments has it been demonstrated with certainty that an animal which has been allowed to become pale in the dark at room temperature can be made to darken by exposure to low temperatures without admission of light. The only experimental evidence we have been able to find which does furnish such a proof is that of SMITH (1929). His determinations were performed upon excised pieces of the skin of *Anolis equestris*. It was discovered by HADLEY (1928) that the excised skin of this species (not, apparently, of any other species of *Anolis*) continues to respond to light and darkness by expansion and contraction of the melanophores. SMITH placed three such pieces in " the shade " where they maintained a green colour. On cooling the solutions in which the pieces were immersed, one turned brown at 9° C., another at 8° C., and the third at 6° C.

Chameleons become pale in the dark at any temperature above zero (C.). Temperatures below zero were not tried.

A normal and a blind chameleon were placed in a refrigerator at night.

Time.	Normal.	Blind.	t° C.
min.			
0	dark	green	17
10	pallor...	pallor...	1
30	pallor...	pallor...	1
60	pallor...	pallor...	1

When taken out the animals were comatose. Fifteen minutes later they had recovered.

Similar results were obtained with many animals in an ice chest at a temperature of 5-6° C. The observations were made both during the day and at night, there being, of course, no light inside the ice chest. In all cases the animals reached a condition of maximum pallor.

In view of these facts no general statement concerning the effect of low temperature upon reptilian pigmentary effectors can be made in the present state of knowledge.

The melanophores in the excised skin of *Anolis equestris* expand in the dark at temperatures below 9° C. The chameleon becomes pale in the dark at all temperatures down to zero. The evidence for *Phrynosoma blainvillei*, *P. cornutum*, and *Anolis carolinensis* has been shown to be inconclusive. It is to be regretted that the response to low temperatures of intact individuals of *Anolis equestris* has not been determined.

In spite of the by no means unequivocal nature of the evidence available on this issue, recent authors unanimously regard it as proven that cold is one of the agencies causing darkening of reptilian skin. Smith (1928), for example, states that " there is a large body of evidence at hand showing that in the reptiles heat produces a contraction and cold an expansion of the melanophores. . . ." Careful perusal of the literature leaves one with the impression that there is a general tendency to the view that the proposition that heat causes melanophore contraction has as a natural corollary the converse proposition that cold causes melanophore expansion, and that, therefore, since the effect of heat is easily and certainly established, the effect of cold is scarcely in need of independent demonstration. Such a view is not only unjustified, it is also dangerously misleading. It is difficult to understand how such an idea has crept into the literature of colour response when it is entirely absent from the physiology of other contractile mechanisms. If we regard the expanded state of the melanophore as the relaxed condition—and there is every reason to so regard it in reptiles (see p. 49) and possibly also in fishes—then the contraction under the influence of high temperatures is analogous to heat rigor of muscle or of cilia (Gray, 1928, p. 78), and is a phenomenon which raises no new physioloigcal issues. The belief that low temperatures can cause melanophore expansion is physiologically far less orthodox, and involves issues of far-reaching theoretical importance. The effect of low temperatures can only be discussed intelligibly with reference to the co-ordinating mechanism of pigmentary control, for since the unstimulated melanophore is already expanded (*i.e.*, relaxed), it is obvious that exposure to low temperatures cannot *cause* expansion, it can only antagonize or inhibit the stimulating effect of some agency which at normal temperatures induces contraction. This distinction was recognized by Smith (1928) who demonstrated very clearly the important principle that heat may affect melanophores of fishes directly, and may also exert an influence upon the pigmentomotor mechanism, and that the end effect is not necessarily the same in the two cases. The issue thus resolves itself into two questions : (*a*) can low temperatures antagonize the effect of any agent capable of causing contraction of *denervated melanophores*, and (*b*) can low temperatures cause darkening of *intact animals* kept under conditions which at room temperatures are known to promote pallor ?

We are aware of no observations in the literature of nerve and muscle physiology which suggest the possibility that low temperature may inhibit a normal response to stimulation. The data collected by Snyder (1908) and by Kanitz (1915) show that the rate of propagation of the nervous impulse, the spontaneous activity of plain and heart muscle, the response of striped muscle to stimulation of the motor nerve all obey

the general laws of the effect of temperature upon physiological processes. In no case has it been found for any temperature range that $Q_{10} = \infty$. If this conclusion is correct, then it follows as a general rule that any excitable tissue must eventually respond, however slowly, to its normally effective stimulus at all temperatures at which it remains alive.

The claim that low temperature abolishes the response of melanophores to normally effective stimuli either in the denervated preparation or in the intact animal implies a theoretical exception of such fundamental importance that it can only be accepted under the pressure of overwhelmingly conclusive evidence. From what has been said above it is clear that such evidence does not exist as far as reptiles are concerned. Without entering into a lengthy discussion of the literature on fishes, amphibia, and crustacea, it may be stated that there is no general agreement concerning the effect of low temperatures on the pigmentary effectors of these groups, from which it is legitimate to conclude that here again no finally conclusive evidence exists.

In view of these considerations and of the new data presented in this paper, we feel that the conclusion is justified that, although high temperatures undoubtedly cause contraction of reptilian melanophores, there is no reason to suppose that the normal pigmentary activity of reptiles is in any *qualitative* way modified by low degrees of temperature.

Part III.

Are Reptilian Chromatophores Independent Effectors?

It has already been pointed out that the chameleon and several other lizards respond locally to light and darkness. When a small area of the skin is more brightly illuminated than the rest of the animal, that area shows a greater degree of darkening. In the chameleon the localization is extraordinarily precise. If a small piece of copper sheeting cut into the shape of a Y is held close to a chameleon exposed to sunlight so as to throw a sharp shadow on the animal's skin, after two minutes a "print" is obtained, the shaded region being paler than the unshaded. The question then arises as to whether this response is a reflex, or whether the chromatophores react directly to light. Brücke (1852) investigated this problem very thoroughly. His work has been almost completely overlooked by recent authors. Nevertheless, his evidence and conclusions appear to us to be unexceptionable.

Brücke showed that (*a*) excised skin, or skin separated from the underlying tissue, always turned black, (*b*) section of the spinal cord did not abolish the response to light and darkness, but (*c*) when the cord anterior to the point of section was destroyed, the skin turned black anterior to the cut, but remained pale behind. This posterior portion still displayed normal chromatic reactions.

Bert (1875), whose communication consists only of a summary of conclusions, without experimental details, states: "Les rayons lumineux appartenant à la region

bleu-violet du spectre agissent directement sur la matiere contractile des corpuscules, pour les faire mouvoir et s'approcher de la surface de la peau." In the absence of any clue as to the manner in which this conclusion was reached, it is impossible to attach much weight to it. KELLER (1895) also envisaged the possibility that there may be a direct stimulation of the chromatophores by light, but adduced no new evidence. FUCHS (1914) regarded all the evidence on this question as inconclusive. REDFIELD (1918) concluded that light acts directly upon the melanophores of *Phrynosoma*. This view has been very generally adopted in recent literature. HOGBEN (1924) accepted it, but in his subsequent work on the chameleon (HOGBEN and MIRVISH) he found that when the spinal cord was cut, " the exclusion of light only resulted in pallor anterior to the point of section," an observation which we have been unable to confirm (see p. 52). From this it was concluded that " the light (and heat) reactions are not wholly independent of the C.N.S." HADLEY (1928) showed that the excised skin of *Anolis equestris* responds to light and darkness by melanophore expansion and contraction. The work of REDFIELD and HADLEY is quoted in this connection by PARKER (1930) who accepts their evidence for the direct response of melanophores to light and concludes that " a similar condition will probably be found in many other vertebrates, though how general it may prove to be is still to be discovered."

Since modern opinion has been so strongly influenced by REDFIELD'S work, it is necessary to examine the data on which he based his conclusions. His experimental findings were the following :

1. " Pieces of skin placed in Ringer's solution show no melanophore reactions."

2. Four different cuts were made through the body wall in four animals in such a way that " had all the cuts been made upon a single animal a part of the skin would have been completely severed from the rest of the body." In the dark all these animals showed uniform pallor. From this REDFIELD concluded that " since this contraction cannot be attributed to a nervous reflex, the conclusion must be drawn that light acts directly upon the melanophores of the horned toad."

3. The sciatic nerves were cut, groups of spinal nerves were severed, and cuts were made through the body wall. " In no case have these operations altered the reactions of the melanophores of the isolated region in any way. *The responses of the melanophores to direct stimulations and to hormones evidently suffice to bring about all ordinary melanophore reactions without the aid of nerves which connect with these cells directly.*" [Italics inserted.]

4. Stimulation of spinal nerves produced absolutely no effect.

5. Stimulation of sciatic nerve " produced a very clear contraction of the melanophore pigment of the left leg," though not in all cases.

6. Section of spinal cord at level of the thirteenth vertebra had no effect on the melanophore reactions to light and darkness.

7. In some cases such a spinal transection caused pallor anterior and darkening posterior to the cut.

8. After the removal of the adrenals from such a preparation the mouth was electrically stimulated. "*The back anterior to the point of transection of the spinal cord*, the fore limbs and all the lateral scales *became very distinctly paler*." [Author's italics.]

From this REDFIELD drew the conclusion that "*the melanophores are co-ordinated by two distinct mechanisms, the adrenal secretion and the direct action of nerves. Either mechanism alone is capable of causing the melanophore pigment to contract.*" (Author's italics.]*

It is impossible to escape the impression that these results are extremely contradictory. To begin with, the two passages quoted in italics are clearly inconsistent. Secondly, if stimulation of the sciatic nerves or of the central nervous system after removal of the adrenals causes melanophore contraction, how is one to account for a negative result on stimulation of spinal nerves? Lastly, if denervated skin still shows the normal reactions to light and darkness, there appears to be no reason why pieces of skin in Ringer's solution should fail to react, nor is it possible to account for the fact that spinal transection in some cases caused pallor anterior and darkening posterior to the cut.

In view of these considerations, one is justified in regarding the evidence of REDFIELD for the direct reactivity of the melanophores of *Phrynosoma* to light as inadequate. There remain only the observations of HADLEY on *Anolis equestris*. Since it is emphasized by this worker that this is the only species of *Anolis* where the pigment cells in excised skin respond to light, it is necessary, until more comparative data have been collected, to regard this as an exceptional rather than a typical case.

In the present investigation on the chameleon the observations of BRÜCKE have been fully confirmed. Any skin separated from the underlying muscle becomes black and remains black permanently. The reactions of excised skin are shown in the following protocol:

Three pieces of skin were cut from the flanks of a decapitated chameleon.
All three turned black in from one to two minutes.
One piece was put into normal saline, one into Ringer's solution, and one was spread out on a glass slide to which it adhered by its under surface.
All three were placed in the dark room, at 17° C.
After one hour all three were still pitch black.
Faradic stimulation of the under surface of each of the three pieces caused a striking pallor.
When stimulation ceased the pieces began to darken, and after two minutes were black again.

The observations show that the tissue was alive at the end of the experiment. The pigment cells in excised skin remain in the condition of extreme relaxation both in darkness and light. This result cannot be ascribed to ionic effects since skin in saline and in Ringer behaved in the same way as dry skin.

* REDFIELD's evidence on the role of the adrenals will be examined in a later section (see p. 47). Here it is desired only to draw attention to his evidence on the question of the direct reactivity of the melanophores to light.

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The most complete proof is furnished by the following experiment:

A chameleon was decapitated. The body cavity was cut open by a longitudinal dorso-lateral cut running just to the right of the spinal column from the neck to the cloaca. The body was cut across in the region of the cloaca, and the viscera, including the heart and dorsal aorta, were removed. The preparation was spread out on a wad of cotton soaked in Ringer. The whole operation was performed in the dark room, with the aid of a photographic safe light on an animal that was maximally pale.* The preparation was alternately exposed to daylight and darkness with the following results:

Time after operation.	Exposure to	Condition at end of exposure.	
		Right Side.	Left Side.
min. sec. min. sec.			
0 00 to 0 30	Darkness	Medium darkening ...	Pallor.
0 30 to 2 30	Daylight	Black...	Darkening.
2 30 to 12 30	Darkness	Black...	Yellow.
12 30 to 15 30	Daylight	Black...	Dark green.
15 30 to 25 30	Darkness	Black...	Yellow.
25 30 to 28 00	Daylight	Black...	Dark green.
28 00 to 33 00	Darkness	Black...	Yellow.
33 00 to 35 30	Daylight	Black...	Dark green.
35 30 to 40 30	Darkness	Black...	Yellow-green.
40 30 to 43 00	Daylight	Black...	Dark green.
43 00 to 53 00	Darkness	Black...	Yellow-green.
53 00 to 55 30	Daylight	Black...	Dark green.
55 30 to 65 30	Darkness	Black...	Pale green dorsally.
65 30 to 68 00	Daylight	Black...	Dark green.
68 00 to 78 00	Darkness	Black...	Yellow-green dorsally, the rest dark green.
78 00 to 80 00	Daylight	Black...	Dark green.
80 00 to 100 00	Darkness	Black...	Dark green.

Figs. 16 and 17, Plate 5, illustrate this experiment. For nearly an hour the melanophores of the left side, *i.e.*, the intact side, continued to respond to darkness by contraction and to light by expansion. The melanophores of the right side, *i.e.*, the transected side, expanded fully 2–3 minutes after the operation, and remained in this condition permanently, irrespective of the conditions of illumination. In this preparation the influence of the vascular system is excluded. Both sides are equally deprived of oxygen and of circulating hormones. *The conclusion is unavoidable that the response of the pigment cells of the chameleon to light is dependent upon the integrity of spinal reflex arcs.*

By means of such a preparation it is further possible to confirm the conclusion of Hogben and Mirvish that the pigmentary effectors are innervated by autonomic fibres.

A dorso-lateral preparation was made. It was left in the dark for ten minutes and then exposed to light to make sure that the intact side was responding normally. Under a dissecting micro-

* This precaution, however, is not obligatory.

scope the sympathetic chain of the intact side was completely removed. The result was that the "intact" side rapidly became as black as the transected side, and remained so even with the total exclusion of light.

This observation leads to the further conclusion that *the neurones involved in the reflex arcs pass through the white and grey rami and the ganglia of the sympathetic chain.*

Part IV.

The Co-ordination of the Normal Responses of the Chameleon to Light and Background.

From the experiments described in the foregoing sections it is evident that the normal chromatic responses of the chameleon are conditioned by the functional activity of two separate, and easily separable, receptor fields: the dermal and the retinal photoreceptors. The response to stimulation of the dermal receptors is best seen in animals whose retinal receptors have been eliminated by section of the optic nerves. Such animals attain the same degree of pallor in the dark as normal animals, figs. 11 and 12, Plate 4. When exposed to light they darken irrespective of the quality of the background. The degree of darkening is roughly proportional to the intensity of illumination.

In the chromatic behaviour of intact chameleons the activity of the retinal receptors is integrated with that of the dermal ones, and results in the background responses described in Part I of this paper. The behaviour of blind and intact animals is similar only in the dark and on a dark background in strong diffuse light, and, of course, under the influence of heat. In a weak light on any background and in strong light on a light-scattering background blind and intact animals behave quite differently, as Plate 4 clearly shows.

The daily rhythm. The visual responses provide an alternative interpretation for a phenomenon which has been observed by several investigators. *Amphibolorus barbatus*, according to de Grijs (1899) darkens in the early morning sunlight, and then turns pale again at midday. Redfield (1918) observed a similar rhythm in *Phrynosoma*. This animal is pale at night, becomes dark in the early morning, but "during the heat of midday the melanophore pigment contracts again." In the evening the animals are again dark. It has been unanimously assumed that this phenomenon is due to the antagonistic action of light and heat, and Redfield's observations are cited by Parker (1930) in support of the theory which has been advocated by several authors that the daily rhythm of colour changes in lizards serves the function of thermoregulation. There can be no doubt that sufficiently high temperatures do cause contraction of reptilian chromatophores, but the view that this effect is of bionomic importance must be accepted with caution. So far as the chameleon is concerned it is very doubtful whether, under natural conditions, this animal would ever be found exposing itself to sunlight of sufficient intensity to cause heat contraction of the chromatophores. On very hot days chameleons conceal themselves deep in the

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shade of the foliage. Further, in our experience, when chameleons are experimentally exposed to temperatures sufficiently high to cause heat pallor, this condition is invariably associated with strong manifestations of distress, the animals struggle violently to escape, and, when prevented from doing so, they soon succumb. Nevertheless, a daily rhythm occurs which has no connection with temperature, but which is entirely explicable in terms of light intensity and background. The data in figs. 2 and 3 lead to the following expectations :

	Normal animals on white background.	Blind animals on any background.
Darkness of night	Pale ..	Pale
Twilight of sunrise	Dark ..	Pale
Bright Daylight	Pale ..	Dark
Twilight of sunset	Dark ..	Pale

These responses are identical with the daily rhythm observed by REDFIELD in *Phrynosoma*. They have been frequently observed in chameleons kept in cages in the laboratory. The presence of blind animals in the cage provides unexceptionable evidence that light and not temperature is the causal agent in the reaction. Indoors, the daily rhythm varies in detail according to the conditions of illumination. In a room on the west side of the building the normal animals may remain dark and the blind animals pale throughout the morning, and the reverse conditions may become established only when the sun strikes the windows in the afternoon. On dull days when the sky is heavily overcast the normal animals may remain dark all day, and the blind animals correspondingly pale. On occasions, also, both normal and blind animals may be of intermediate shade. Evidently the conditions in a cage (made of planks and wire netting, and containing twigs and leaves) approximate to those of a " light-scattering background," because when, during a bright afternoon, the pale chameleons are taken out and placed in a black dish, they become dark.

If the daily rhythm observed by REDFIELD in *Phrynosoma* is explicable along lines similar to these, then the important conclusions follows that *Phrynosoma* displays the same remarkable reversal of the white background response in weak light as the chameleon. At present, however, no such conclusion is warranted, since REDFIELD does not record observations on the daily rhythm of blind animals, nor does he define the conditions under which his observations were made. He does not state whether the animals were indoors or in the open, and makes no reference to the season of the year. The latter is a curious omission for during at least six months out of the twelve " the heat of midday " in Massachusetts is not intense.

The mechanism of nervous control.—The work of BRÜCKE, KELLER, BERT, and HOGBEN and MIRVISH has established beyond question the fact that the chromatophores of the chameleon are under the control of the nervous system. HOGBEN and MIRVISH have

further shown that the innervation is derived from the autonomic system. REDFIELD'S conclusions on this question have already been quoted (see p. 42). His work on the role of the adrenals in the pigmentary responses of *Phrynosoma* constitutes the exclusive evidence on which PARKER bases his recent claims that both nervous and endocrine factors are concerned in reptilian colour response (for quotations see p. 28). In view of the importance ascribed to REDFIELD'S work it is necessary here to draw attention to a contradiction in his experimental data which leaves his main conclusion almost without foundation. On p. 43, we have quoted REDFIELD'S experiment in which after removal of the adrenals, stimulation of the mouth resulted in distinct pallor. This was advanced as evidence for "the direct action of nerves." His claim that adrenaline may be the agent causing pallor is supported by a similar experiment in which "*after the removal of the adrenal glands no contraction of the melanophore pigment resulted, although the mouth was stimulated in the same way for twenty minutes.*" [Author's italics.] Clearly, only one of these two observations is true. Epinephrectomy either does or does not abolish the response to faradic stimulation of the mouth. REDFIELD'S data lend equal support to either of these alternatives.

These considerations, when viewed in relation to the recent investigations of HOGBEN and MIRVISH, justify the statement that to-day no positive evidence exists that the adrenal organs are in any way concerned in the colour responses of reptiles. If, then, the whole gamut of pigmentary responses is co-ordinated through the nervous system, the mechanism of this nervous control must be exceedingly intricate. In order to gain some insight into the nature of this mechanism the following facts must be considered:

(*a*) Denervated melanophores expand, and can only be caused to contract by heat, electrical stimulation, pharmacological agents experimentally applied (*e.g.*, adrenaline), and possibly certain combinations of electrolytes. This expansion occurs when the skin is separated from the underlying tissues, or when the spinal nerves are cut (*e.g.*, dorso-lateral preparation) or when the sympathetic chain is removed, or when the spinal cord is destroyed by pithing.

(*b*) Electrical stimulation of the central nervous system causes generalized pallor. When the spinal cord is transected pallor occurs only on that side of the cut which is stimulated (HOGBEN and MIRVISH). Electrical stimulation of individual spinal nerves causes a localized response in the corresponding area of the skin.

In this connection, certain details have come to light which are of importance because failure to recognize them may lead to serious misconceptions. All the evidence supports the expectation that section of a nerve would result in the paralysis of a corresponding area of the skin, which would remain permanently black while the rest of the animal would respond to light and darkness, and to background in the normal way. We have found it possible to cut spinal nerves by a method which involves a minimum of operative disturbance. The chameleon is opened, under ether, by a

median ventral incision extending from in front of the cloaca to the pectoral girdle. When the viscera are moved to one side the spinal nerves can be seen with the aid of the dissecting microscope lying in each segment underneath the peritoneum, which as in some other reptiles is black. It is not difficult to make a small tear in the peritoneum, and to cut the exposed nerve without hæmorrhage and without damage to any other structures. The body cavity is then stitched up. If care is taken to leave no opening into the body, the animal begins to breathe soon after the operation, and rapidly recovers. Animals thus operated upon have survived for more than two months.

When a spinal nerve was transected in this manner, we found to our great surprise that no black patch appeared on the animal's skin ; the whole animal responded normally to light and darkness. The operation was repeated with the same result. Further investigation revealed the cause of this apparently inconsistent observation. The area of skin innervated by a spinal nerve is overlapped by the area innervated by the

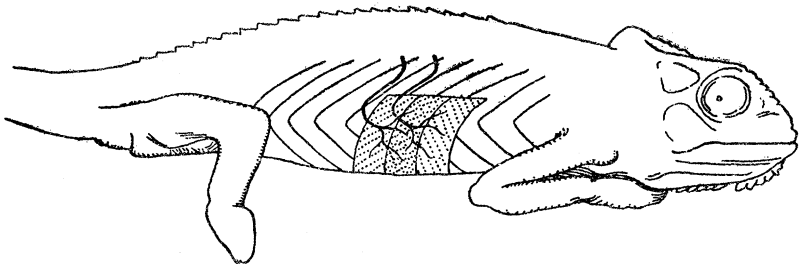

FIG. 4.—The dermal areas supplied by two consecutive spinal nerves (ventral branches only). The position of the ribs is indicated.

nerve next in front and behind in such a way that every spot on the skin, and presumably every melanophore, receives fibres from two consecutive spinal nerves. The distribution of spinal nerves (ventral branch only) to the skin is shown in fig. 4. There are several ways of demonstrating this. If in a decapitate and eviscerate preparation a spinal nerve is electrically stimulated and the area of pallor is marked with coloured thread, it is found to extend over two segments, from the middle of the segment in front of the one whose nerve was stimulated, to the middle of the third segment in front. When the next nerve is stimulated and the area of pallor marked, the anterior half of this area is found to overlap the posterior half of the first area, and so on. Similarly, when in such a preparation four or five *alternate* spinal nerves are cut along the length of the trunk, electrical stimulation of the cut anterior end of the spinal cord causes general pallor of the skin, but if then one or more of the intervening nerves which had been left intact are cut, a black patch appears which no longer responds to central stimulation. Chronic operations have fully confirmed this observation. Four consecutive nerves have been cut on the left side and four alternate nerves on the right side in the same animal, and in one operation. The animal survived for more than two months, a patch of skin on the left side turned black, the right side

was unaffected. Fig. 13, Plate 5, shows an animal with three consecutive spinal nerves cut. It will be noticed that the black patch extends only over the ventral half of the flank. It is to be presumed that the skin of the dorsal half is supplied by the dorsal branch of the spinal nerve, which cannot be approached in so small an animal.

The black patch persists only for about three weeks. It then becomes paler and eventually, after six or eight weeks, yellow. Such an animal, when put on a black background appears very dark, with the denervated patch yellow. In no experiment has the denervated area regained the power of responding to light and darkness. The causes of this change are obscure. Possibly it is due to necrosis of the denervated tissue—a sort of rigor mortis—since after death chameleons are usually maximally pale.

It must be emphasized that the innervation of the skin revealed by our experiments is not a "double innervation" in the sense in which this term is usually employed. It was Bert (1875) who first made the totally undocumented statement that the chromatophores of chameleons are controlled by a contracting and expanding nervous mechanism analogous to the vasodilator and vasoconstrictor apparatus. This idea has been taken up by others, but no evidence has been advanced in support of it. The type of innervation that we have found may be described perhaps as anatomically double but physiologically single, because, as far as the evidence goes, each melanophore receives a fibre from each of two consecutive spinal nerves, and both are pigmentomotor, *i.e.*, contracting, fibres. We can make no suggestions as to the significance of this curious arrangement. One feels that it ought to have some relation to the mottled effect which we have called "contrast" (p. 35), but no interpretation along these lines has suggested itself.

To return now to the question of the co-ordinating mechanism. Since nerve section results in maximal expansion of the denervated melanophores, and nerve stimulation causes maximal contraction, the expanded melanophore must be deemed to be in a state of relaxation, and the contracted in a state of excitation. This was the conclusion reached by Brücke, and there can be no doubt that it is correct. But we have shown that the darkening of illuminated skin is a reflex phenomenon, which means that the stimulation of the dermal photoreceptors by light causes a *relaxation* of the pigmentary effectors. This fact only becomes intelligible on the assumption that the response to stimulation of the dermal photoreceptors which, for brevity, we will call the dermal response, is due to reflex inhibition. And since in the dark, *i.e.*, in the absence of photic stimulation, the melanophores are maximally contracted, one is forced to conclude that they are maintained in this condition—in a state of tonic contraction, as it were—by the agency of the pigmentomotor neurones. This interpretation was also considered by Brücke, but with some degree of scepticism for the reason that he knew of no parallel example of such inhibition among spinal reflexes. To-day the objection is no longer valid. Brücke further envisaged the possibility that darkness may act as a stimulus in a manner analogous to the action of cold in

exciting the pilo-motor apparatus. That this is a false analogy needs no demonstration. It may be supposed, however, that the shadow responses of certain invertebrates (*e.g.*, tubicolous worms) are germane to this issue. Against such a supposition it is only necessary to point out that chameleons remain pale in darkness indefinitely, and that embryos are always white in the uterus (our species is viviparous), and darken immediately after birth or when they are removed by operation. There can be no question of a shadow response before birth.

The view that the dermal response is a reflex inhibition gains support from a consideration of the time relations of the responses to darkness and to light. PARKER and STARRATT (1904) found that at 20° C. *Anolis carolinensis* became green in the dark in about 20 minutes and brown in the light in $4\frac{1}{4}$ minutes. For the chameleon the times are remarkably similar. Fig. 2 shows that blind animals brought from darkness into daylight become equilibrated in about five minutes. Although we have not determined the reverse response with the same degree of precision (because it is impossible to record animals in the dark at frequent intervals without illuminating them for a considerable proportion of the time), it may be safely stated that chameleons

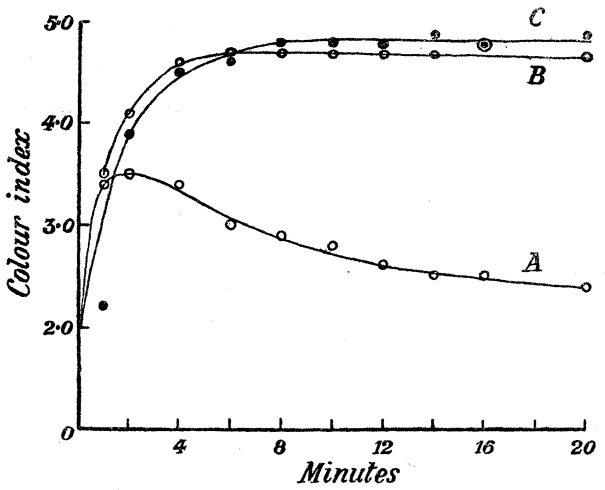

FIG. 5.—Curves taken from figs. 2 and 3. Curve A. Normals on white in daylight. Curve B. Blind on white in daylight. Curve C. Normals on black in lamplight.

do not attain maximum pallor in the dark in less than 15 minutes. This difference in times is compatible with the view that melanophore expansion is due to a release from sympathetic control, while contraction is due to the establishment of a state of tonic excitation.

If the dermal response is a reflex inhibition, what is the nature of the retinal responses to background? This is a problem of great complexity and one for the solution of which sufficient data do not at present exist. Nevertheless, a feasible interpretation may be suggested. Fig. 5 shows the time curves, taken from figs. 2 and 3, of blind animals on white in daylight and normal animals on black in lamplight. The curve

for normal animals on white in daylight is also here reproduced. It will be observed that curves B and C show no significant difference. The state of darkening attained is the same, and the velocity of melanophore expansion is the same. Now the curves for blind animals in fig. 3 show that in lamplight these animals remain pale, which means that this light is not sufficiently intense to stimulate the dermal photoreceptors. This being so, it follows that the darkening of the normals on black in lamplight (curve C, fig. 5) is due almost exclusively to impulses proceeding from the *retinal* photoreceptors, whereas the darkening of blind animals in daylight (curve B) is due exclusively to impulses proceeding from the *dermal* photoreceptors. The fact that the stimulation of two different sets of receptors has exactly the same end effect strongly suggests that the co-ordinating mechanism in the two is similar, and since the dermal response is an inhibition, the retinal response to a light absorbing background must be a similar inhibition.

When, however, the response of normal animals on white in daylight (curve A) is considered, it is apparent that the conditions are such that the dermal response should occur (cf. curve B). It is *prevented* from occurring by the stimulation of the retinal receptors by light from a light-scattering background, and the animals remain pale. It seems reasonable to regard this phenomenon as an inhibition of inhibition. If this suggestion is provisionally accepted, the shape of curve A becomes intelligible. It is consonant with physiological principles that a primary inhibition should become temporarily established before a secondary one asserts itself, if the stimuli for both are applied simultaneously. The steep upward slant of the curve would then represent inhibition resulting from the stimulation of the dermal photoreceptors. It is noteworthy that this steep portion coincides with the other two primary inhibition curves shown in the figure. After a minute or so the secondary inhibition comes into play, and the pigmentomotor apparatus, released from inhibiting impulses, induces the proximal migration of the melanophore pigment—slowly, as it does when an animal is taken from light to darkness.

It is a very remarkable fact that an exactly similar response of animals brought from darkness into light on a white background has been observed by PARKER and LANCHESTER (1922) in fishes. Fundulus, when taken from a dark box, were pale. They were placed on a white background in full daylight. "After a few seconds the fishes from the dark box, though they were on a light background, grew dark, reaching a maximum in about half a minute. They then remained dark for about five minutes, after which they took on the light tint normal for their surroundings." If this similarity is real, and not merely apparent, then it implies that the mechanism of pigmentary control in Fundulus and the chameleon is identical in every essential respect. Such far-reaching conclusions, however, must await the results of further investigation.

We will refrain from the attempt to fit the curious reversal of the white background response in weak light, fig. 3, curve A, into the neurological scheme which we have

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suggested. This response is still insufficiently investigated. There is reason to believe that an even weaker light than the one which we have employed would furnish the most favourable conditions for it. It may be pointed out, however, that this response occurs when the illumination is not strong enough to evoke the dermal response. If the white background response in strong light is, as we have suggested, an inhibition of inhibition, then it is obvious that this response cannot occur in weak light, for an inhibition which does not exist cannot be inhibited. Concerning the nature of what occurs in its place, we will not venture to speculate.

It is possible to demonstrate the dermal and retinal responses in one and the same animal. Figs. 14 and 15, Plate 5, show an animal whose spinal cord was transected at the 8th vertebra. It was shown by HOGBEN and MIRVISH that such a preparation responds to electrical stimulation of the mouth by pallor anterior to the cut only. They stated, moreover, that "in all cases where the region posterior to the point of section failed to respond to stimulation of the mouth, the exclusion of light only resulted in pallor anterior to the point of section." This observation was based on animals which were kept alive after spinal transection "for more than a week." We have kept three such animals for three months. It is true that for a week or two they do not respond to darkness by complete pallor. But after about a fortnight their responses appear again, and they now behave like normal animals anterior to the cut, and like blind animals posteriorly. Fig. 14 shows the animal on a white background in lamp-light. It is dark in front and pale behind. The anterior half of the body corresponds to the condition of a normal animal, fig. 8, Plate 4, the posterior half to that of a blind animal (fig. 10). Fig. 15 shows the same animal on the same background in daylight. The response is reversed. It is now pale in front and dark behind. The anterior half corresponds to the condition of a normal animal fig. 7, Plate 4, the posterior half to a blind animal (fig. 9). The darkening of the posterior half of the body in daylight is less extreme than for the blind animal, suggesting an analogy with the increased tone of certain muscles associated with decerebrate rigidity in mammals.

Conclusion.—The possibility of formulating a general theory of reptilian colour response is still remote. The work of CARLTON (1903) on *Anolis carolinensis* and of HADLEY (1928, 1929) and SMITH (1929) on *Anolis equestris* shows that this genus is fundamentally different from the chameleon. Both these lizards, like the chameleon, were found to become pale (green) in the dark, and to darken (become brown) on exposure to light. Moreover, both agree with the chameleon in that melanophore contraction in the dark is relatively slow (*A. carolinensis* 20 minutes; *A. equestris* 15–18 minutes) and melanophore expansion is relatively rapid (*A. carolinensis*, 4 minutes; *A. equestris*, 1 minute). There, however, the comparison ends. CARLTON found that excised skin turns *green* both in the dark and in the light. Moreover, such skin may be made to assume the brown colour by gentle mechanical stimulation. From this he reasonably inferred that the state of contraction is the resting condition of the melanophores. The same conclusion must be drawn from HADLEY'S work on

A. equestris. Excised skin of this animal placed in physiological salt solution continued to respond for three hours to weak and strong light by pallor and darkening respectively. A curious fact upon which HADLEY failed to comment was that whereas the response times of whole animals were as quoted above, the times for excised skin were: green to brown 30–40 secs., brown to green 12–15 secs. Not only are these times much shorter, but their relation to each other is reversed. In whole animals melanophore expansion takes place much more rapidly than contraction, in excised skin contraction is the more rapid. Still more puzzling are the data on the effect of temperature on the excised skin of this species, SMITH (1929). We have already referred to this work as constituting the only clear evidence for the expansion of reptilian melanophores at low temperatures under conditions of illumination that promote melanophore contraction. Now if in this species the proximal position of the pigment is the resting (*i.e.*, relaxed) state of the melanophore, as must be inferred from the fact that the denervated skin is green in the absence of photic stimulation, it appears from SMITH's data that *heat induces a state of relaxation*, and cold promotes the opposite state (excitation). It is not unreasonable to assume, on purely theoretical grounds, that an *independent effector* which is found to respond to photic and thermal stimulation, should respond to both kinds of stimuli in the same way. With the melanophores of *A. equestris* this is apparently not so; they expand in light and are contracted by heat. In the absence of other experimental data it is difficult to suggest an explanation. The experiment with the dorso-lateral preparation (p. 47), if carried out on these species, would go far towards elucidating the main difficulties that at present exist.

In view of the fact that all reptiles whose pigmentary responses have been physiologically investigated belong to one class, namely, the Lacertilia, and since, further, all observations point to the conclusion that the reactions to environmental conditions are superficially alike, namely, pallor in darkness and on exposure to high temperatures and darkening in light, it is difficult to believe that they are physiologically totally dissimilar, and that an elaborate apparatus of tonic innervation and central inhibition should exist in one species and be quite unrepresented in another.

In the present state of knowledge one can do no more than hope that when the background responses of other reptiles have been thoroughly investigated, and the problem of direct reactivity finally solved, the materials for the formulation of a general theory of reptilian colour change will be obtained.

We wish to express our sincere thanks to Mrs. ANNE STEPHENSON for the photography and the drawing of the text figures in this paper.

Summary.

1. In strong diffuse daylight chameleons show a response to background. They become dark on a black background, and pale on a white one.

2. Blind animals darken in the light. This response has been shown to depend upon the integrity of spinal reflex arcs.

3. The time relations of these responses have been determined.

4. The threshold for the retinal photoreceptors is lower than for the dermal ones.

5. In weak light the white background response is reversed. The animals become dark.

6. Low temperatures above 0° C. have no effect upon the normal response of chameleons to darkness.

7. The work of other investigators on low temperatures is discussed, and theoretical considerations are advanced against the view that low temperatures can abolish the normal responses to light and darkness.

8. The following theory of nervous co-ordination is developed :

(*a*) The melanophores are maintained in a state of tonic contraction by pigmentomotor fibres belonging to the autonomic system.

(*b*) Stimulation of the dermal photoreceptors by light inhibits the tonic innervation.

(*c*) Stimulation of the retinal photoreceptors by light from a light absorbing background similarly inhibits the tonic innervation.

(*d*) Stimulation of the retinal photoreceptors by light reflected from a light-scattering background causes an inhibition of the inhibition due to the simultaneous stimulation of the dermal photoreceptors.

9. It is suggested that the " daily rhythm " of colour changes may be interpreted in terms of the white background response in strong and weak light, without reference to temperature.

10. It is pointed out that, in the present state of knowledge, the materials for the formulation of a general theory of reptilian colour change do not exist.

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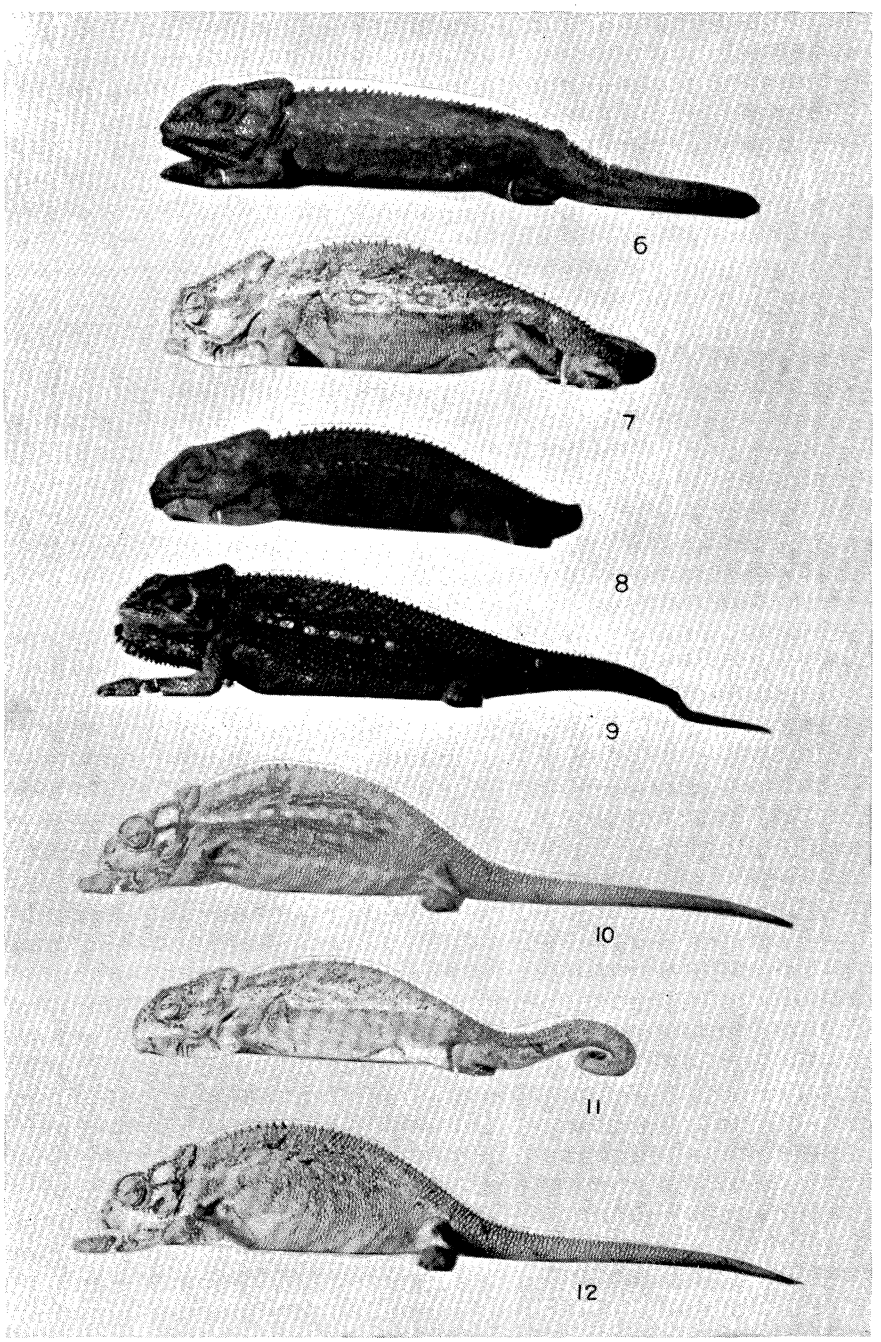

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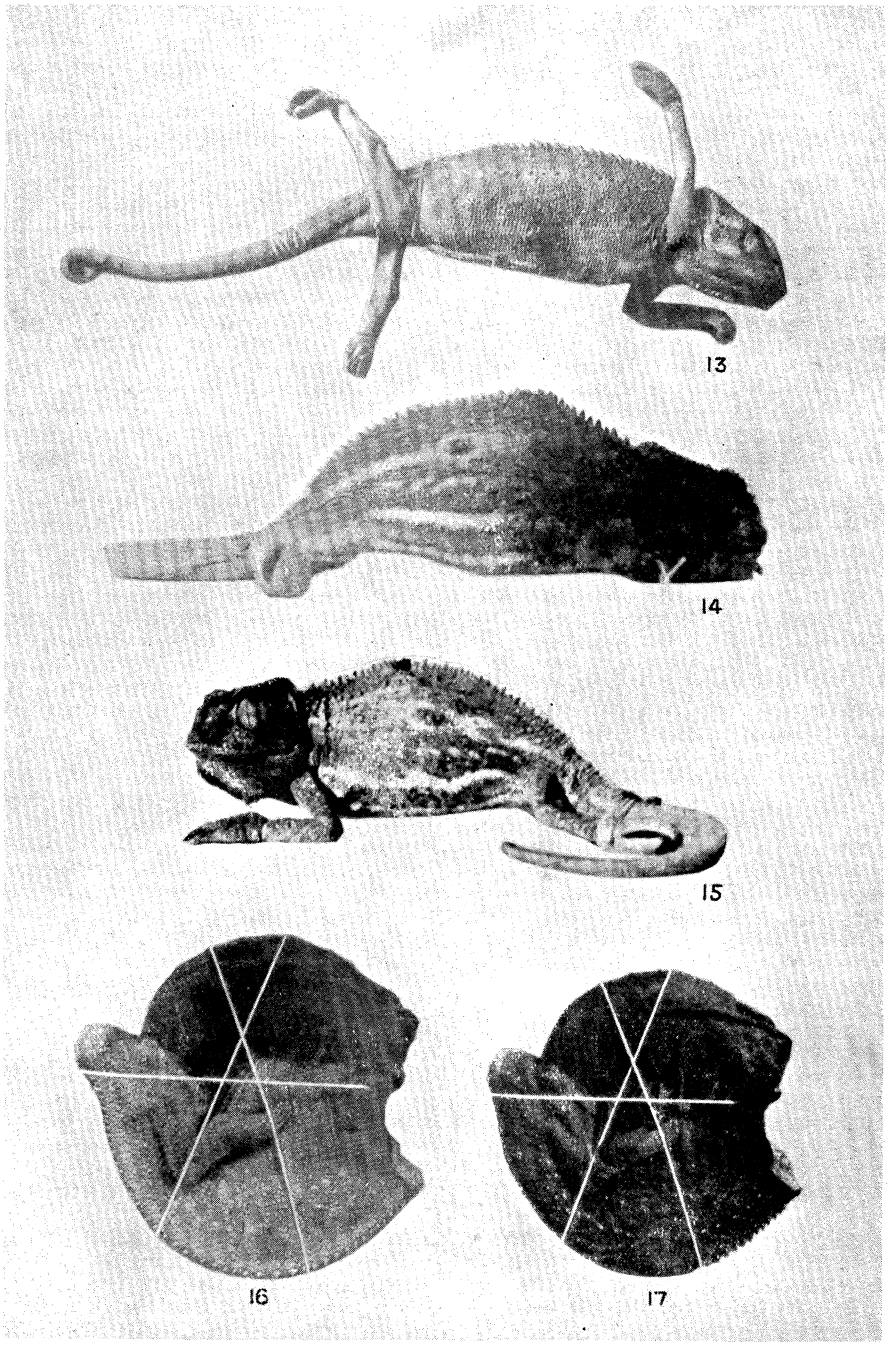

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DESCRIPTION OF PLATES.

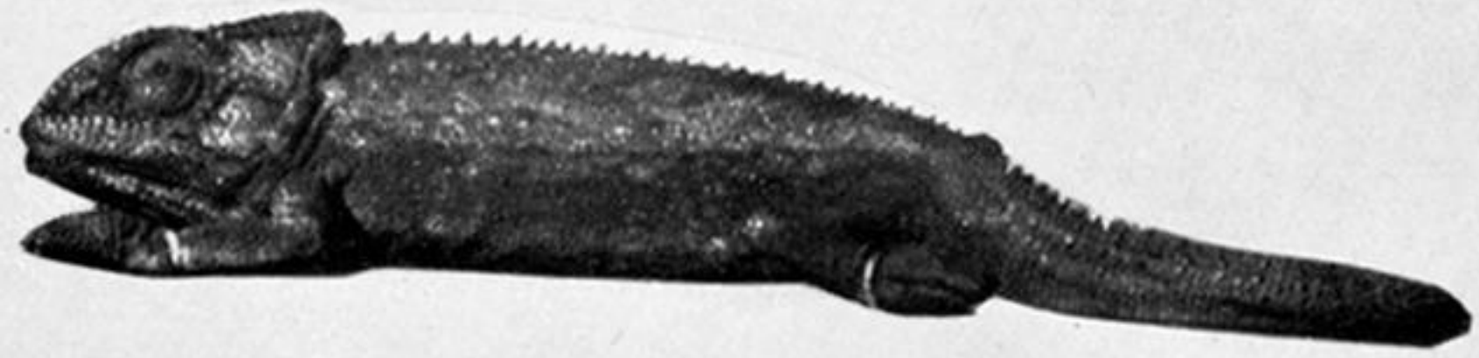
PLATE 4.

FIG. 6.—A chameleon on black cardboard in daylight.
FIG. 7.—The same animal on white cardboard in daylight.
FIG. 8.—The same animal on white cardboard in lamplight. Flashlight photograph.
FIG. 9.—A blind animal on white cardboard in daylight.
FIG. 10.—The same blind animal on white cardboard in lamplight. Flashlight photograph.
FIG. 11.—The same animal as in figs. 6-8 in darkness. Flashlight photograph.
FIG. 12.—The same blind animal as figs. 9 and 10 in darkness. Flashlight photograph.

PLATE 5.

FIG. 13.—A chameleon with three consecutive spinal nerves cut, in darkness. Flashlight photograph. Sixteen days after operation.
FIG. 14.—A chameleon with the spinal cord transected. Three months after operation. The animal is on white cardboard in lamplight. Flashlight photograph.
FIG. 15.—The same animal as fig. 14 on white cardboard in daylight.
FIG. 16.—Dorso-lateral preparation after being left in the dark for 10 minutes. Flashlight photograph. The intact side is yellow-green, the transected side is black.
FIG. 17.—The same preparation after a few minutes exposure to daylight. Both sides are black.

* See note on preceding page.



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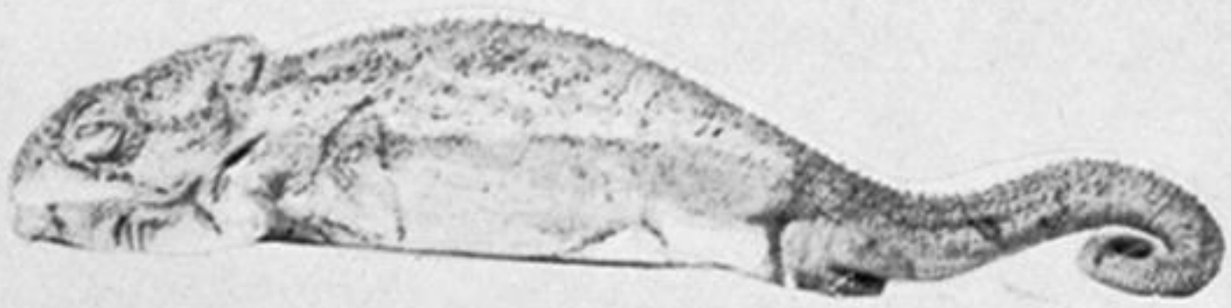

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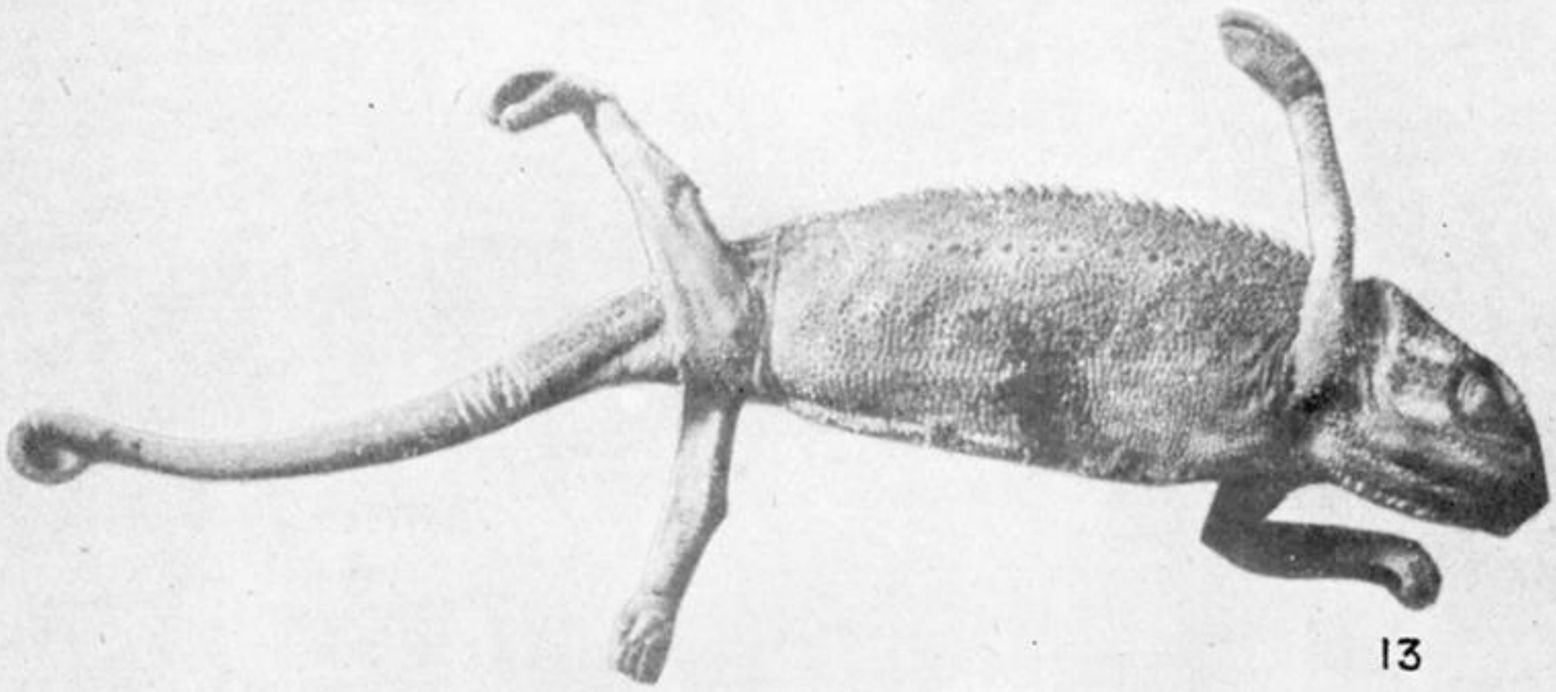

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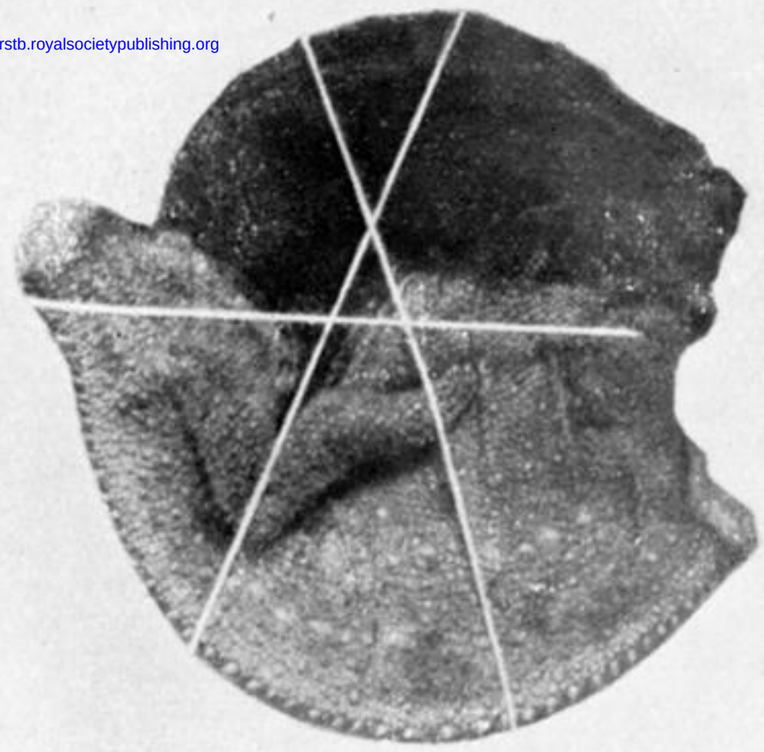

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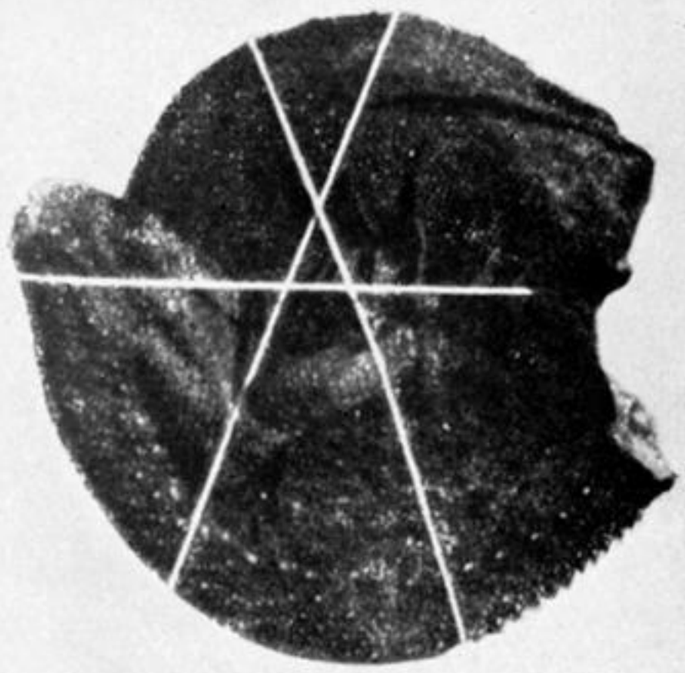

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Plate 5.

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